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Antimicrobial Resistance in *Neisseria gonorrhoeae*: Proceedings of the STAR Sexually Transmitted Infection-Clinical Trial Group Programmatic Meeting

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Abstract

The goal of the Sexually Transmitted Infection Clinical Trial Group's (STI-CTG) Antimicrobial Resistance (AMR) in *Neisseria gonorrhoeae* (*NG*) meeting was to assemble experts from academia, government, non-profit and industry to discuss the current state of research, gaps and challenges in research and technology as well as priorities and new directions to address the contsinued emergence of multi-drug resistant *NG* infections. Topics discussed at the meeting, that will be the focus of this article, include AMR *NG* global surveillance initiatives, the use of whole genome sequencing (WGS) and bioinformatics to understand mutations associated with AMR, mechanisms of AMR, and novel antibiotics, vaccines and other methods to treat AMR *NG*. Key points highlighted during the meeting include: (i) US and International surveillance programs to understand AMR in *NG*. (ii) The US National Strategy for combating antimicrobial resistant bacteria. (iii) Surveillance needs, challenges and novel technologies. (iv) Plasmid- and chromosomally-mediated mechanisms of AMR in *NG*, (v) Novel therapeutic (e.g., sialic acid analogs, FH/Fc fusion molecule, monoclonal antibodies, topoisomerase inhibitors, fluoroketolides, LpxC inhibitors) and preventative (e.g., peptide mimic) strategies to combat infection. The way forward will require renewed political will, new funding initiatives and collaborations across academic and commercial research and public health programs.

Keywords

antimicrobial resistance; *Neisseria gonorrhoeae*; sexually transmitted infections

Introduction

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae (NG)* continues to be a serious threat to global public health. Although the use of dual antimicrobial therapy is highly effective, increasing reports of *NG* infections with cephalosporin- and azithromycin-reduced susceptibility raise serious concerns regarding the durability of current treatment recommendations (1). Although nearly 400,000 *NG* cases were reported in 2015, the United States Centers for Disease Control and Prevention (CDC) estimates about 820,000 total infections occur annually due to the underreporting of asymptomatic undetected cases (4). While AMR in *NG* continues to be a concern both in the US and globally, current nucleic acid-based amplification testing methods cannot measure antimicrobial susceptibility. Therefore, enhanced molecular diagnostics that distinguish among *NG* infections with antimicrobial resistance versus reduced susceptibility versus susceptibility are needed to help guide antibiotic treatment. The development and use of new bioinformatics tools, in conjunction with new technologies like whole genome sequencing (WGS) methods to identify AMR *NG*-associated mutations may resolve this issue at a global level. Understanding the mechanisms of AMR in *NG* may also help guide the development of new treatment and preventative modalities. The STAR STI-CTG held a programmatic meeting in Silver Spring, Maryland on April 13th, 2017 titled: “Antimicrobial Resistance (AMR) in *Neisseria gonorrhoeae* (*NG*)”. Experts from academia, government, non-profit and industry reviewed the current state of research, gaps and challenges in research and technology as well as future research and public health directions.

Surveillance Programs to Understand AMR

The STD Surveillance Network (SSuN)—To complement routine notification data, CDC established the STD Surveillance Network (SSuN) in 2005. In select jurisdictions, laboratory test results are collected from STD clinic attendees along with epidemiological data from a random sample of persons with gonorrhea (5). Data are representative of *NG* testing of the rectum, urethra, cervix and pharynx. In some SSuN jurisdictions, more than 50% of reported gonorrhea cases occurred among MSM in 2015 (3). In other jurisdictions, cases in women and heterosexual men were more common, suggesting epidemic differences that may require different prevention and control approaches. In STD clinics participating in SSuN, the *NG* positivity rate among MSM tested for gonorrhea was over 5% and was elevated among HIV-infected MSM (e.g., ~17% of HIV-infected MSM tested had rectal gonorrhea

Gonococcal Isolate Surveillance Project (GISP)—Established in 1986 to monitor *N. gonorrhoeae* antimicrobial susceptibility and inform treatment guidelines, GISP is a collaboration between the CDC, clinical sites, and regional laboratories (6). Urethral specimens for culture and antimicrobial susceptibility testing are systematically collected from consecutive men with urethritis each month at participating STD clinics according to a

standardized protocol; limited epidemiological data are locally abstracted from medical records and later merged, by CDC, with antimicrobial susceptibility data. GISP is designed for long-term surveillance of susceptibility trends; data are not available in a timely manner to inform clinical management and public health response. While GISP is aimed at surveillance of *NG* in men, the Enhanced Gonococcal Isolate Surveillance Project (eGISP) was later created (2015) to strengthen surveillance of gonorrhea susceptibility and increase state and local capacity to detect and monitor *NG* in women and from extra-genital sites.

During 2006–2016, the proportion of GISP isolates with reduced susceptibility (minimum inhibitory concentration (MIC) ≥0.125 μg/ml) to ceftriaxone) remained low (less than 0.5%) (3;7). The proportion of isolates with reduced azithromycin susceptibility (MIC ≥2.0 μg/ml) increased from 0.6% in 2013 to 3.6% in 2016 (6;7). Recently, of particular concern, there were four GISP isolates collected in Hawaii that had elevated MICs to both azithromycin (MICs ≥16.0 μg/ml) and ceftriaxone (MICs= 0.125 μg/ml) (8). Isolates collected through GISP continue to show reduced susceptibility to antimicrobials no longer recommended as first line regimens; preliminary data for 2016 indicate that approximately 40% isolates had some resistance to penicillin, tetracycline, and/or ciprofloxacin.

Based on the approximately 820,000 gonococcal infections that occur each year in the U.S., it was predicted that in 2011 about 246,000 infections either were resistant or had decreased susceptibility to at least one antibiotic; 11,480 had reduced susceptibility to cefixime (MIC ≥0.25 μg/mL), 2,460 reduced susceptibility to azithromycin (MIC ≥2.0 μg/mL) , and 3,280 reduced susceptibility to ceftriaxone (9). *NG* isolates with decreased susceptibility to cephalosporins are often resistant to other classes of antibiotics as well (10–12). Although those susceptibility trends are concerning, it is important to note that there have been no clinical treatment failures in the US with the current recommended therapy of 250 mg ceftriaxone and 1g azithromycin.

International Gonococcal Antimicrobial Surveillance Program (GASP)—To support international surveillance of gonococcal resistance, the World Health Organization (WHO) founded GASP in 1990 (13). GASP currently has participating countries in Africa, the Americas, the Eastern Mediterranean, Europe, South East Asia, and Western Pacific. Different countries have different approaches to AMR in *NG.*

From 2009 to 2014, the total number countries reporting to GASP increased from 56 to 77 but there was considerable variation between WHO regions reporting. Of the 77 countries reporting to GASP, 66% reported isolates with any resistance/decreased susceptibility of *NG* to cephalosporins (cefixime or ceftriaxone), 81% with any resistance/decreased susceptibility of *NG* to azithromycin, and 97% with any resistance/decreased susceptibility of *NG* to ciprofloxacin, for at least 1 year from 2009 to 2014 (15). Notably, there are large gaps in data on AMR *NG* in Africa, Central America (extending up to Mexico), and the Middle East with adjacent countries in Asia. Currently, differences in the US and European guidelines for MIC interpretation create challenges to combining disparate country reports. Hence, as countries continue to develop robust surveillance programs and report MIC values, strategies to combine and compare such data need to be further examined and standardized.

Multidrug resistant (MDR) and extensively drug resistant (XDR) forms of *NG* have been identified globally, including isolates from Japan (11;16–19), Hawaii (8;16;20) and England (21). The WHO defines MDR-*NG* as isolates with reduced susceptibility or resistance to either extended spectrum cephalosporins (ESC) or spectinomycin (i.e., category I antibiotics), plus two or more of macrolides, fluoroquinolones, penicillins, tetracycline, aminoglycosides and carbapenems (i.e., category II antibiotics) (22). XDR-*NG* are defined as isolates with decreased susceptibility or resistance to category I antibiotics and three or more category II antibiotics (22). Resistance or reduced susceptibility to cephalosporins (i.e., ceftriaxone) due to the emergence of strains with mosaic *pen*A alleles has been noted in the aforementioned countries. Reduced susceptibility to macrolides, such as azithromycin, have also been noted (22–26).

Questions persist about how to either implement or enhance surveillance, especially in low- and middle-income countries, how best to report AMR in *NG*, what the cost/benefit is for validating treatment failures in low- and middle-income countries, whether older antibiotics can be employed again with new molecular diagnostics to predict susceptibility and how to validate various treatment guidelines from around the world. Treatment guidelines for *NG* must also be updated based on in-country surveillance data as many countries continue to use ciprofloxacin as a recommended first-line therapy (24). One success of the GASP program includes updated treatment guidelines for *NG* in countries such as Argentina, Chile, Bolivia, Colombia, Cuba, Uruguay and Venezuela.

The Recent Public Health Response

U.S. National Strategy for Combating Antimicrobial Resistant Bacteria—The U.S. National Strategy for Combating Antibiotic-Resistant Bacteria, released in September of 2014, put forth five overarching goals: slowing the development of resistant bacteria and preventing spread of resistant infections; strengthening surveillance; advancing the development and use of rapid and innovative diagnostics; accelerating of research and development for new antibiotics, therapeutics and vaccines; and improving international collaboration (9). Reflecting the designation of *NG* as one of three urgent antibiotic resistance threats (27), the National Strategy included a national target of maintaining the prevalence of ceftriaxone-reduced susceptible *NG* at <2% through 2020 and beyond. The National Action Plan, released in March 2015, outlined a roadmap for implementing the National Strategy.

In Fiscal Year 2017, Congress appropriated $167 million to CDC to support implementation of the National Strategy through CDC's Antibiotic Resistance Solutions Initiative. While the Initiative is broad-based, multiple activities focusing on *NG* are included; selected activities are described below. To strengthen surveillance, the Antibiotic Resistance Laboratory Network (ARLN) was created. Seven state public health laboratories serve as regional laboratories to conduct AMR testing of multiple pathogens as well as specialized testing of clinical specimens. Four of the laboratories conduct agar dilution testing of *NG* for GISP and other enhanced surveillance platforms. Integration of WGS of *NG* is planned.

Using Antibiotic Resistance Solutions Initiative funding, CDC also implemented the Strengthening US Response to Resistant Gonorrhea (SURRG), a collaboration between

CDC and participating jurisdictions to establish local capacity to rapidly detect and respond to AMR in selected local jurisdictions (27). Jurisdictions participating in SURRG collect specimens for *NG* culture in STD clinics and other healthcare settings, conduct rapid susceptibility testing on all isolates, interview patients infected strains with reduced antimicrobial susceptibility and their recent contacts, and expand data collection to facilitate epidemiological and network analyses. The Antibiotic Resistance Solutions Initiative funding is also strengthening surveillance of *NG* isolates for drug susceptibility patterns in GISP and monitoring of trends of gonorrhea in SSuN.

World Health Organization—In response to the increasing threat of AMR, the World Health Assembly adopted a global action plan on antimicrobial resistance in May of 2015 (28). The WHO's five objectives are: (i) to improve awareness and understanding of antimicrobial resistance through effective communication, education and training; (ii) to strengthen the knowledge and evidence base through surveillance and research; (iii) to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; (iv) to optimize the use of antimicrobial medicines in human and animal health; (iv) to develop the economic case for sustainable investment that takes account of the needs of all countries and (v) to increase investment in new medicines, diagnostic tools, vaccines and other interventions. This action plan emphasizes the need for a coordinated approach leveraging international stakeholders from different disciplines and sectors.

Surveillance Needs, Challenges and Novel Technologies

Surveillance Needs and Challenges—From 2000 to 2010, global antibiotic usage increased by 36% and use of cephalosporins doubled (29), particularly in China and India. Such increases in antibiotic use likely drives the selective pressure for AMR. Although global surveillance efforts (e.g., GISP/GASP) strive to detect AMR in *NG* isolates, one must consider whether those data are being collected with sufficient timeliness to mitigate the risks. Through current surveillance programs, there is a lag in identifying AMR *NG* for clinical decision-making, thereby potentially enabling continued transmission of AMR strains. The process is limited by the constraints of current methods and technologies in growing *NG* isolates, identifying AMR and its mechanisms, and identifying isolates implicated in AMR outbreaks through phenotypic characterization. Greater use of molecular tools for timely and accurate detection of AMR as are applied on other fields of infectious disease surveillance including PCR and DNA sequencing is urgently needed.

Cases of ceftriaxone-reduced susceptible *NG* have been found in pharyngeal specimens (23) (24). Pharyngeal gonorrhea poses multiple challenges due to its asymptomatic nature, ease of transmission and difficulty of treatment. The pharynx may also serve as an *NG* reservoir and incubator of reduced susceptibility because of the frequent presence of commensal non-pathogenic *Neisseria* species. Given that *Neisseria* species are known for DNA uptake and exchange, it is likely that the horizontal transfer of genetic material, including antibiotic resistance genes, in the pharynx leads to AMR *NG* infections

In many regions globally, antibiotics are readily available without a prescription, and those regions are historically known for high levels of antibiotic resistance and have groups of people with high rates of oropharyngeal STIs. Given that environment, the NIH-funded (Fogarty Center) ICON Study in northern Vietnam, enrolled MSM to address the frequency of antibiotic use and any association with antibiotic resistant- or susceptible- pathogenic as well as non-pathogenic *Neisseria* (30). Preliminary results of the ICON Study found 62% of current participants reported antibiotic usage in the prior six months, often without a prescription and some stopped antibiotic usage as soon as symptoms abated. Non-pathogenic *Neisseria* were found in 38 (100%) of 38 clinical pharyngeal specimens, with some samples growing up to four different *Neisseria* species, including *N. gonorrhoeae* and *N. meningitidis*. Next steps of the ICON Study include determining whether different *Neisseria* species have different capacities for acquiring resistance, determining the prevalence of similar genetic components in different resistant strains, and whether *Neisseria* commensals can be used in surveillance to predict tends in *NG* AMR.

Novel Technologies—Advances in genomics might help address AMR through informing the development of molecular diagnostics, identifying outbreaks, advancing the understanding of disease transmission, and through epidemiological/evolutionary inference to guide antibiotic selection. Important AMR-related questions that genomics can help address include: (i) How much resistance is due to clonal spread and/or *de novo* emergence, (ii) To what extent do known genetic resistance mechanisms explain observed phenotype resistance, and (iii) How can the scientific community identify novel mechanisms of resistance.

Reports have shown that increased MICs to extended-spectrum cephalosporins (cefixime MIC ≥ 0.25 μg/mL; ceftriaxone MIC ≥ 0.125 μg/mL) in the U.S. is predominantly associated with the mosaic *penA* XXXIV allele with or without additional specific point mutations in *pen*A (31–34). Quinolone-resistant *NG* has widely spread through predominantly spread of mutations in *gyrA* and *par*C (gyrA-S91F/I, gyrA-D95G, parC-S88P). Reduced azithromycin susceptibility has arisen through multiple mechanisms, with the most common in the U.S. being 23S rRNA mutations (C2611T, and A2059G) as well as mosaic mtr mutations in the mtrR locus (35;36) However, about a third of reduced azithromycin susceptibility (MIC ≥ 2 μg/mL) is not clearly explained by 23S rRNA mutations, by a mosaic mtr locus, by a single base pair deletion in the mtrR promoter or generation of a new promoter for transcription of mtrCDE (36) . Those findings indicate the utility of WGS in developing nucleotide-based molecular diagnostics. However, several limitations are worth noting. First, not all phenotypic resistance is explained by known mechanisms of resistance. Further, the frequency with which novel mechanisms of resistance arise, mixed strain infections occur or how to best screen for such mechanisms or determine the clinical impact of mixed infections is unclear.

Genomic epidemiology can help understand patterns of spread of gonococcal strains and identify local transmission and outbreaks. Examples include tracking the transmission of resistant lineages across geographic and demographic boundaries (35–38) , as well as reconstructing local transmission networks (37;38).

Development of point-of-care (POC) diagnostics to identify drug susceptibility profiles has the potential to impact overall levels of AMR and, as 60% of gonococcal isolates in the US are pan-susceptible, permit reintroduction of older antibiotics into treatment regimens (42–47). While a rapid test for susceptibility is expected to aid in reducing the overall burden of AMR as compared to one that does not detect susceptibility (48), questions remain about how best to deploy these strategies.

Mechanisms of Antimicrobial Resistance (AMR) in *Neisseria gonorrhoeae (NG)*, and Novel Antibiotics and Vaccines to treat AMR *NG*

Mechanisms of beta-lactam antibiotic resistance in NG—There are two genetic sources of antibiotic resistance in *NG*: plasmid-mediated and chromosomally-mediated. In plasmid-mediated resistance, beta-lactam antibiotic resistance occurs due to the expression of an antibiotic modifying protein (e.g., TEM-1-like β-lactamase for penicillin and amino-penicillin (e.g. amoxicillin) resistance [Pen^R]) or a ribosome-protected protein (TetM ribosomal-binding protein that confers tetracycline resistance). □-lactamase does not hydrolyze cephalosporins, so it does not contribute to cephalosporin resistance. However, one amino acid change in the *bla* gene could convert it to produce extended-spectrum β-lactamase (49). In chromosomal-mediated resistance, antibiotic resistance occurs due to *de novo* spontaneous mutations or due to the acquisition of chromosomal mutations via homologous recombination commonly thought to occur from *Neisseria* commensal species. In stepwise resistance, each step is a relatively small increase in resistance, but when multiplied overall, it leads to a large increase in the MIC of a given antimicrobial.

The main difference between Pen^R *NG* strains and cephalosporin resistant ($Ceph^R$)/ cephalosporin-intermediate-resistant strains ($Ceph^I$) is due to the type of mosaic *pen*A allele arising from interspecies recombination (1). It appears that the origin and rapid emergence of $Ceph^I$ strains was due to a single transformation event of a mosaic *penA* allele into existing $Ceph^S/Pen^R$ strains, which to this day persist even though penicillin has not been used for *NG* treatment in decades (50).

In addition to the *penA* allele, the *mtrR* and *penB* determinants contribute additional resistance to β-lactam antibiotics and provide a general permeability barrier for antibiotics (51). The mtrR determinant, caused by mutations either in the promoter region or coding sequence, increases transcription of the divergently transcribed mtrCDE operon that encodes the MtrC-MtrD-MtrE efflux pump (52–54). The increased expression of the pump causes increased efflux of antibiotics from the cytoplasm and periplasm of *NG*. The mutated *pen*B may produce altered forms of the $PorB_{1B}$ porin, the major porin of *NG* (55;56) resulting in a decrease in the influx of antimicrobials through the porin channels. Interestingly, the increase in resistance conferred by *penB* requires the presence of an *mtrR* mutation (57).

Novel Therapeutic and Vaccine Approaches—Novel, non-traditional therapeutic and vaccine approaches to combat multi-drug resistant *NG* infection are currently being investigated. Therapeutic approaches include sialic acid analogs (e.g., chemical therapies) (58–60), FH/Fc fusion molecules (59–61) and monoclonal antibodies (e.g., immunotherapeutic molecules). Vaccine approaches include widely expressed antigens that

are immunogenic (e.g., common lipooligosaccharide epitopes represented by peptide mimics) (62–64) as well as the use of vaccines developed for other *Neisseria* species that may cross-protect against *NG* infections

Non-gonococcal sialic acids can be used to disrupt the natural protection on most gonoccocal organisms. More specifically, endogenous, host mammalian sialic acids are taken up by gonococci *in vivo* and result in protection of the organism from complement-mediated killing whereas non-host sialic acids, derived from alternative sources, do not possess this protective function (complement resistance). With respect to mechanism of action, when alternative sialic acids are administered locally to infected mice, they replace host sialic acid, can be taken up preferentially by gonococci and hasten clearance of bacteria by removing resistance to complement-mediated killing (58–60). Natural and synthetic sialic acids can be mined for candidates that are optimal in eliminating complement resistance and hastening clearance of infecting bacteria.

A fusion protein has been engineered that on the one hand binds to a complement regulator binding site, present on all gonococci, called factor H (FH), and on the other hand possesses an Fc domain that engages complement and kills the organism; thereby, enhancing clearance in the animal model. The FH portion has been altered so as not to bind to human cells thereby avoiding toxicity. FH/Fc fusion protein constructs have been shown to bind to 12/15 different gonococcal isolates, kill 10/15 of these *in vitro* and hasten clearance of 3 different isolates infecting the animal model (59–61). Production of FH/Fc, a fully humanized immunotherapeutic, is being scaled up in tobacco plants and configured for use parenterally and in intravaginal release devices.

Another immunotherapeutic molecule being developed for gonorrhea treatment is the chimeric (mouse/human) 2C7 antibody. The 2C7 antibody has been tested, intravaginally and parenterally, in the mouse animal model (62). The 2C7 antibody is being fully humanized and like FH/Fc, production is being scaled up in tobacco plants and configured for parenteral and intravaginal administration. Because 2C7 antibody and FH/Fc target different sites on the organism, combining their use may be additive.

The 2C7 epitope, against which the 2C7 antibody was developed, forms the basis for a novel gonococcal vaccine (6;62;63;65). The 2C7 epitope is displayed by greater than 95% of clinical isolates; antibodies against the 2C7 epitope are elicited uniformly by women with infection (58;62;66). A 2C7 peptide mimic vaccine was constructed by screening of randomly generated peptides (using a peptide library consisting of $> 10^{12}$ peptides) and identifying peptide(s) recognized by 2C7 monoclonal antibody (52). A multi-antigenic peptide (MAP; octomeric/tetrameric) was fashioned that elicited antibodies directed against the nominal (2C7) epitope, possessed complement-dependent killing against all gonococcal isolates tested and hastened clearance of infection in vaccinated animals (65). Stabilization and scale-up of homogenous peptide (>95% pure) has already been accomplished and current work is aimed at optimizing responses to the peptide mimic vaccine with human-approved adjuvants. While meningococcal group B outer membrane vesicle vaccines have been shown to be immunogenic and efficacious against homologous strains, more recently they have also been found to protect partially against *NG* infection. A retrospective case-

control study of patients seen in New Zealand sexual health clinics revealed that exposure to the outer membrane vesicle meningococcal B vaccine was associated with about 30% reduction in gonorrhea diagnoses (67) . While those novel therapeutic and preventive approaches provide hope in curtailing gonococcal infections, they will require more research and development to deliver an approved, affordable treatment for AMR *NG* that can be brought to the clinic. Meanwhile there are few novel, more traditional antibiotic approaches that are in development.

Novel Therapeutic for uncomplicated NG: Zoliflodacin/ETX0914—The standard CDC and WHO treatment recommendation for gonorrhea requires a minimal efficacy of greater than 95% at any mucosal site (cervix, urine, rectum, pharynx)(12). An optimal treatment would be effective against resistant isolates for both urogenital and extra-genital infection and would be well-tolerated (68). While a single dose therapy would be ideal, single dose therapy versus multi-dose therapy is less a priority than a safe and well-tolerated antimicrobial regimen with efficacy across resistant isolates and all anatomic sites.

Zoliflodacin (Entasis Therapeutics) was developed for the treatment of uncomplicated gonorrhea and is the first drug in a novel class of topoisomerase inhibitors (68). Zoliflodacin has shown potent *in vitro* activity against 100 gonococcal isolates and shows a lack of cross-resistance to other antibiotic classes (69;70). Because its mechanism of action is distinct from fluoroquinolones, it is hypothesized that zoliflodacin will be effective in treating fluoroquinolone-resistant infections. In Phase 1 studies, a single dose of zoliflodacin was well tolerated in healthy adult males and all adverse events were mild/non-serious. No adverse events lead to study discontinuation (ClinicalTrials.gov NCT01929629).

A NIAID-sponsored, Phase 2 study (ClinicalTrials.gov NCT02257918) of zoliflodacin was conducted to assess safety and microbiological cure among 180 subjects with gonorrhea. Out of the 180 subjects enrolled,131 were analyzed as microbiological-intent-to-treat evaluable subjects (71). The number of participants with microbiological cure at urethral or cervical sites in the 2000 mg zoliflodacin, 3000 mg zoliflodacin, and the 500 mg intramuscular ceftriaxone group were 55/57, 54/56, and 28/28, respectively (71). Among 15 patients across the 3 groups with rectal infections, all were cured (71). The number of patients with microbiological cure at pharyngeal site was slightly higher in the patients treated with intramuscular ceftriaxone (4/4) compared with the 3000 mg zoliflodacin group (9/11) (71). Overall, zoliflodacin was well tolerated. Phase 3 studies of zoliflodacin are currently being planned with support from the Global Antibiotic Research Development Program.

Solithromycin—Solithromycin (Cempra, Inc.) is a 4th generation macrolide and the first fluoroketolide. It exhibits *in vitro* activity against a number of urogenital pathogens including *NG* (72;73), *Chlamydia trachomatis* (74), *Mycoplasma spp* (75) and *Ureaplasma spp.* (76). Solithromycin was tested in a Phase 2 urethritis study to assess the eradication of urogenital *NG* (ClinicalTrials.gov NCT01591447) and in a Phase 3 study to assess its non-inferiority versus intramuscular ceftriaxone plus oral azithromycin (ClinicalTrials.gov NCT02210325). The Phase 2 study enrolled 59 subjects, and found 100% eradication across all urogenital, pharyngeal and rectal sites. Solithromycin was associated with

gastrointestinal-related adverse events (77).A follow up Phase 3 study (ClinicalTrials.gov NCT02210325) (N=262) compared solithromycin alone versus standard of care (500 mg intramuscular ceftriaxone [CTX] plus 1 g oral azithromycin [AZI]). In the intent-to-treat analysis Solithromycin was non-inferior to the standard of care (80.5% versus 84.5% cure, respectively) (78). In the microbiologically evaluable population (i.e., patients with a positive baseline culture who returned for evaluation at the TOC visit), treatment success was 91.3% (95/104) for solithromycin recipients, versus 100% (107/107) for CTX/AZI patients. Among the 9 solithromycin patients with a positive TOC culture result, here was no correlation between outcome and solithromycin MIC (range, 0.004–0.25 μg/mL); all baseline isolates were susceptible to solithromycin using CDC criteria for azithromycin (MIC <2.0 μg/ml) (78). Emergence of solithromycin resistance in TOC isolates was not observed. Genotyping of pre- and post-treatment isolates did not demonstrate reinfection with novel strains. Given the absence of baseline or acquired solithromycin resistance and the absence of evidence of reinfection with a novel strain, the investigators surmised that the most likely cause of treatment failure was pharmacokinetic-related, with presumed insufficient duration of drug exposure at the site of infection. It is hypothesized that solithromycin dose adjustment (for instance, a two-dose strategy, over 24 hours) and/or combination treatment strategy with a second antibiotic would result in desired treatment success rates. While other novel therapeutics for NG are currently being investigated, including the triazaacenaphthylene antibacterial agent, gepotidacin, these were not discussed at the programmatic meeting.

Other Means to Treat Gonococcal Infection

Crippling Selective Gene Expression—Understanding the mechanisms of AMR in *NG* can assist in the design of newer antimicrobials and vaccines and provide insights as to the development of compensatory mutations that reverse fitness defects yet maintain resistance. The MtrCDE drug efflux contributes significantly to such resistance and transcriptional control systems modulate levels of efflux pump gene expressions and, as a consequence, levels of antibiotic resistance (75–77). Mutations that increase efflux pump gene expression can adversely impact clinical efficacy of antibiotics. Finally, dampening efflux pump gene expression might allow for return of an old antibiotic (e.g., penicillin) or allow for continued use of current antibiotics.

Strategy to alter Bacterial Membranes - Lipid A Enzymes—Lipid A is a component of bacterial outer membranes and is essential for cell viability of nearly all Gram-negative bacteria. Current investigations are aimed at evaluating whether small molecule inhibitors (e.g., TU-514, CHIR-090, LPC-067) of LpxC, an essential gene for *NG*, can be used to target *NG* infections. LpxC Inhibitors (e.g., LPC-169, LPC-174, LPC-201, LPC-211) have been shown to overcome existing antibiotic resistance (unpublished data). The investigators also looked at the efficacy of LPC-211 in mouse models against a specific ceftriaxone resistant strain of *NG*. While the investigators have found such inhibitors to work well to treat *NG*, improvements are still needed to file an IND application.

Next Steps: Research and Technology Gaps and Challenges

AMR NG Research Gaps and Challenges—In 2016, seven patients in Hawaii were found to be infected with strains demonstrating high-level azithromycin resistance (MICs ≥16.0 μg/ml) and elevated MICs to ceftriaxone (MICs=0.125 μg/ml) (8;20). While those are rare, the chances of combined azithromycin and ceftriaxone-reduced susceptibility are growing. A recent report from China found about 3% of *NG* isolates with dual ceftriaxone decreased susceptibility and azithromycin resistance (1). Molecular studies have found that there is considerable variability in the mutations associated with azithromycin-reduced susceptibility (34;79). An important question to consider is how the scientific community can best monitor reduced susceptibility to azithromycin in regions across the globe? One strategy may be to increase AMR surveillance programs like GISP/GASP globally and expand the collection of non-urogenital specimens. (16;80).

Previously, gonorrhea was treated using antimicrobial monotherapy; specific antimicrobials were recommended based on clinical trial results and subsequent antimicrobial susceptibility trends. The use of dual therapy potentially introduces more complexity into decisions about treatment recommendations. The value of dual therapy to prevent AMR is only a theoretical argument at present; investigations of whether using two or more antibiotics at one time slows the development of resistance to either drug would advance the field. To that end, murine modeling studies may play an important role in addressing such questions in addition to understanding host-microbe interactions. Creating antimicrobial susceptibility testing matrices that include different doses for each drug may help to determine if the combination of drugs are synergistic or antagonistic and may help to address the aforementioned question of resistance. While several synergy studies of drugs against *NG* have been published, little to no antagonism or synergy has been noted (81–83). As new antimicrobial agents, such as those discussed previously, and diagnostics become commercially available in coming years, questions about how to select the most effective drug combinations, weighing both clinical efficacy and impact on resistance, should be addressed with additional synergy studies.

Syndromic Management and AMR NG—Syndromic management continues to be the principal approach for STI treatment in low- to middle-income countries because of its simplicity and affordability (38–44). Syndromic management is based on the identification of clinical symptoms (or signs) with resultant indications for treatment rather than the making an etiological diagnosis using laboratory methods. While inexpensive and fast, the shortcomings of syndromic management include a lack of specificity and substantial overuse of antibiotics. Syndromic management may greatly contribute to AMR in *NG*. Another problem with syndromic management is that it does not address those with asymptomatic infections and is therefore unlikely to impact the burden of infection. Implementing rapid point-of-care detection of *NG* as a first step in the diagnosis of gonorrhea and potentially even more the valuable the point-of-care detection of *NG* with specific antimicrobial susceptibility could profoundly impact and slow the emergence of AMR in *NG* (84).

Conclusion

In conclusion, while dual therapy remains highly effective, existing isolates of infections with dual reduced susceptibility to extended-spectrum cephalosporins and - azithromycin threaten the current recommended treatment for gonorrhea. Thus, new antimicrobials and innovative prevention and control strategies are urgently needed. Approaches to reduce AMR *NG* include the ongoing development and careful introduction and stewardship of novel antibiotics, expanded AMR monitoring and better use of genomics combined with companion diagnostics to rapid identify infection and specific antimicrobial susceptibility, novel vaccine approaches, and special incentives for commercial diagnostic and therapeutic developers.

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